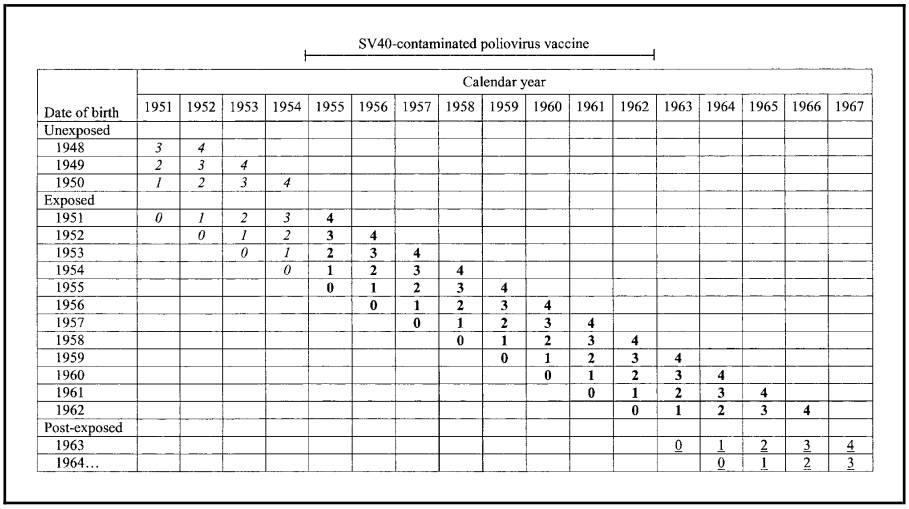
Re: Cancer Incidence in Denmark Following Exposure to Poliovirus Vaccine Contaminated With Simian Virus 40

In a recent article, Engels et al. *(1)* analyzed cancer incidence in Danish birth cohorts that differed in their exposure to simian virus 40 (SV40)-contaminated poliovirus vaccine. Engels et al. also performed a subgroup analysis of cancer incidence in children who were aged 0–4 years before, during, and after the period of vaccine contamination. The authors concluded that SV40 exposure is not associated with an increased incidence of mesothelioma, intracranial tumor, ependymoma, choroid plexus tumor, non-Hodgkin's lymphoma, or leukemia. However, in our opinion, an increased incidence of ependymoma related to SV40 exposure was observed, and the results for mesothelioma and osteosarcoma were inconclusive.

Because newborn animals are reported to be more susceptible to SV40 oncogenic effects than adult animals *(2),* we contrasted the reported crude incidence rates of ependymoma in the exposed-as-infants cohort (0.51) and the exposed-as-children cohort (0.35) and obtained a crude relative risk of developing ependymoma of 1.46 (95% confidence interval [CI] = 1.06 to 1.95; P = .02). Surprisingly, Engels et al. did not report this comparison. The fact that the relative risk was increased is further strengthened by the results of the subgroup analysis of ependymoma in children aged 0–4 years shown in Table 2 of the Engels et al. article *(1)* that reported a relative risk of 2.59 (95% CI = 1.36 to 4.92) for the exposed cohort versus the unexposed cohort. In addition, we also calculated the relative risk of developing ependymoma for the post-exposed cohort versus the unexposed cohort and found that it was 2.48 (95% CI = 1.84 to 3.27; P = .001).

Engels et al. *(1)* also evaluated ependymoma incidence by calendar year (Fig. 2 of their article). They reported that seven cases of ependymoma occurred in 1964, which was after the poliovirus vaccine was cleared of SV40 contamination, four of which occurred in children aged 0–1 years. The authors excluded the possibility that some of the



Date of birth	Calendar year																
	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967
Unexposed																	
1948	*3*	*4*															
1949	*2*	*3*	*4*														
1950	*1*	*2*	*3*	*4*													
Exposed																	
1951	*0*	*1*	*2*	*3*	**4**												
1952		*0*	*1*	*2*	**3**	**4**											
1953			*0*	*1*	**2**	**3**	**4**										
1954				*0*	**1**	**2**	**3**	**4**									
1955					**0**	**1**	**2**	**3**	**4**								
1956						**0**	**1**	**2**	**3**	**4**							
1957							**0**	**1**	**2**	**3**	**4**						
1958								**0**	**1**	**2**	**3**	**4**					
1959									**0**	**1**	**2**	**3**	**4**				
1960										**0**	**1**	**2**	**3**	**4**			
1961											**0**	**1**	**2**	**3**	**4**		
1962												**0**	**1**	**2**	**3**	**4**	
Post-exposed																	
1963													0	1	2	3	4
1964…														0	1	2	3

Fig. 1. Children at risk, by calendar year, age, and date of birth. Data refer to Fig. 2 of Engels et al. *(1).* Ages in italic refer to children born before the polio vaccination period. Ages in bold refer to children vaccinated with simian virus 40 (SV40)-contaminated poliovirus vaccine. Underlined ages refer to children born after the SV40 vaccination period.

seven cases observed in 1964 were associated with vaccination of the mother during pregnancy, which is a known possible risk factor *(3).* It is also important to point out that, in the analysis by calendar year from 1963 through 1966, some of the exposed children were counted as person-years (Fig. 1) and as cases in the unexposed incidence rates. Indeed, seven cases of ependymoma observed in the exposed cohort were attributed to the post-exposed period (i.e., 1963–1966). Moreover, the trend in incidence rates observed by calendar year can be attributed to the effect of SV40-contaminated poliovirus vaccine, particularly if we take into consideration the necessary latency period. Hence, a role for SV40 in the observed increase in incidence of ependymoma, particularly for exposures in the first years of life or during pregnancy, may be hypothesized.

For mesothelioma, the age of the unexposed cohorts during the follow-up period ranged from 0 to 33 years. Because it was impossible to compare cancer rates in the exposed and unexposed cohorts after 33 years of age, the authors should only affirm that incidence of mesothelioma in the first 33 years of life did not increase among the exposed cohorts with respect to the unexposed cohort. However, this neoplasm is extremely rare before age 40 years. For osteosarcoma, where the follow-up started in 1978, the ages of the exposed-as-child and the unexposed cohorts during the follow-up period were 26–51 years and 7–33 years, respectively. Similar to the general age limitations for occurrence of mesothelioma, osteosarcoma is extremely rare after age 25 years. In addition, the lack of incidence data in the period between 1962 and 1978 does not allow a definitive conclusion about possible increases in incidence in the years immediately after the vaccination period.

In summary, estimation of data not available (i.e., in the periods before and after the follow-up) through regression modeling, particularly in the period most relevant to cancer risk evaluation (i.e., 1962–1978), may bias risk estimation. Moreover, we believe that comparison of incidence rates among the available age strata is the best option for definitive assessment of cancer incidence in individuals exposed to SV40.

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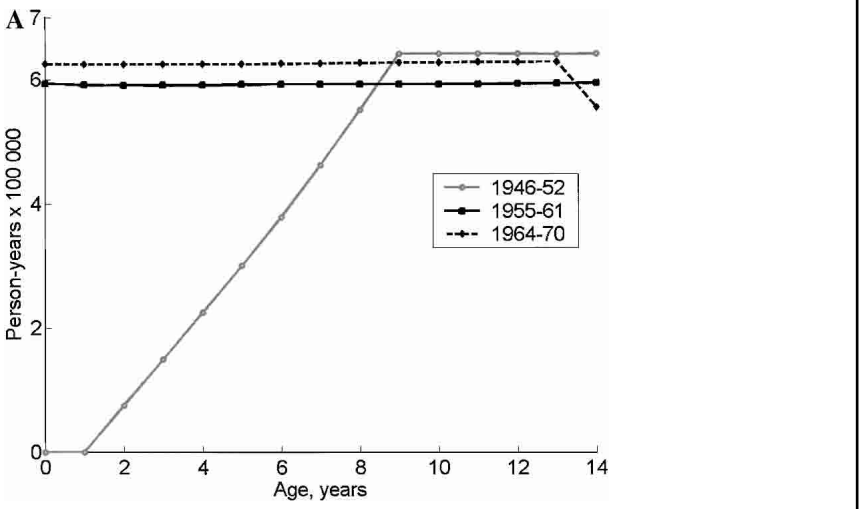
RESPONSE

We thank Puntoni et al. for their comments on our recent article *(1);* however, we disagree that our data demonstrate a relationship between early-life exposure to simian virus 40 (SV40)-contaminated poliovirus vaccine and ependymoma incidence. Puntoni et al. calculated a crude relative risk (RR) of 1.46 (95% confidence interval [CI] = 1.06 to 1.95) comparing the 1955–1961 birth cohort (exposed as infants) with the 1946–1952 birth cohort (exposed as children). However, this comparison is of uncertain relevance, because it contrasts two SV40-exposed cohorts rather than the exposed-as-infants and unexposed cohorts, as we did in our article *(1).* Furthermore, when we calculated a crude relative risk for this comparison (RR = 1.44), our confidence interval was wider than what Puntoni et al. report, regardless of the method we used to calculate it (95% CI = 0.86 to 2.42, assuming asymptotic normality and 95% CI = 0.84 to 2.55, using an exact method). These two confidence intervals, which appropriately incorporate the uncertainty in measured ependymoma incidence in both birth cohorts *(2),* do not indicate an increased incidence in the exposed-as-infants cohort.

More importantly, this crude comparison does not accurately capture the relationship between SV40 exposure and cancer risk, because the three birth cohorts differ in age composition. Specifically, the data for the 1946–1952 birth cohort do not cover years before 1955, when poliovirus vaccine was introduced. Thus, reflecting birth years for individuals in this cohort, there were no data for 0- to 1-year-old children and relatively few data for 2- to 8-year-old children (Fig. 1, A). Overall, ependymoma incidence decreased with age (Fig. 1, B). Therefore, the paucity of



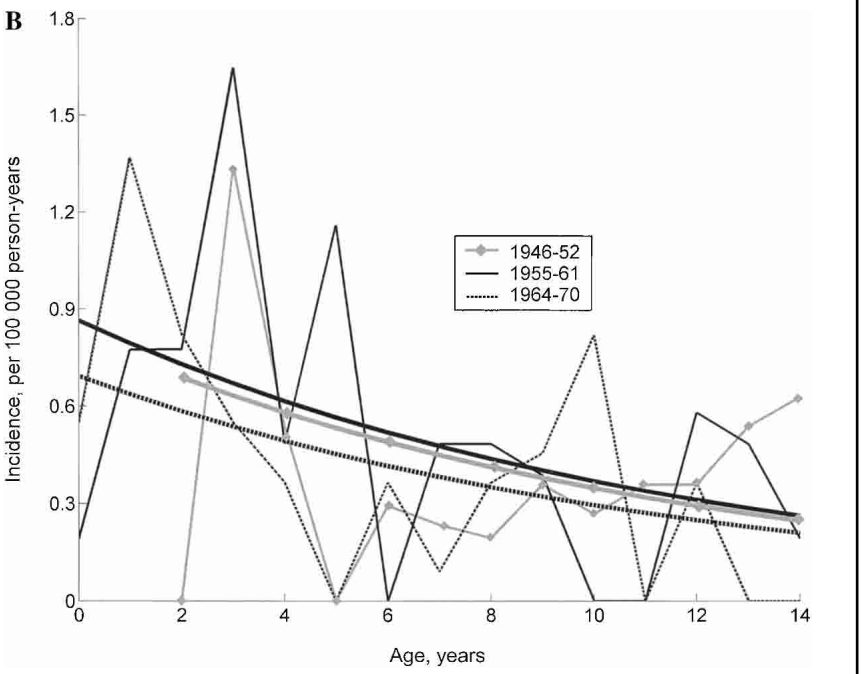


Fig. 1. A) Amount of follow-up time (person-years) by age, for three Danish birth cohorts with varying exposure to simian virus 40 (SV40)-contaminated poliovirus vaccine. The three birth cohorts are 1946–1952 (exposed to SV40-contaminated poliovirus vaccine as children, beginning in 1955) (**gray line with circles**), 1955–1961 (exposed to SV40-contaminated poliovirus vaccine as infants) (**black line with squares**), and 1964–1970 (unexposed) (**dashed line with diamonds**). Data for the 1946–1952 birth cohort include only calendar years beginning in 1955, the year when poliovirus vaccine was introduced in Denmark. Hence, there were no data for this cohort before age 2 years and limited data for ages 2–8 years. **B**) Ependymoma incidence by age, for three Danish birth cohorts with varying exposure to SV40-contaminated poliovirus vaccine. The three birth cohorts are 1946–1952 (exposed to SV40-contaminated poliovirus vaccine as children, beginning in 1955) (**gray lines with diamonds**), 1955–1961 (exposed to SV40-contaminated poliovirus vaccine as infants) (**black lines**), and 1964–1970 (unexposed) (**dashed lines**). Observed incidence (**thin lines**) and fitted estimates (**thick lines**), which were calculated using a Poisson model that used regression splines to smooth age-specific incidence [*see (1)*], are shown. There were no data for the 1946–1952 birth cohort before age 2 years and limited data for ages 2–8 years (*see* **A**).

data for infancy and early childhood in the 1946–1952 birth cohort strongly biases crude comparisons with this cohort. Indeed, when we adjusted for age using regression splines *(1),* the 1955–1961 and 1946–1952 birth cohorts did not differ in ependymoma incidence (age-adjusted RR = 1.06, 95% CI = 0.60 to 1.87; $P = .84$).

Contrary to the comments by Puntoni et al., our further analysis of ependymoma incidence in 0- to 4-year-old children over time also provided no evidence of an effect of SV40 infection *(1).* In that analysis, we carefully considered the birth years of children in each period, so that for the 1963–1966 period, only children born before 1963 were categorized as SV40-exposed [Table 2 of our original article *(1)*]. Puntoni et al. point out that ependymoma incidence was higher in the post-exposed period compared with the unexposed period. However, this increase in incidence among children who never received SV40-contaminated vaccine suggests a broad increase in ependymoma risk over time, rather than an effect attributable to the time-limited use of SV40-contaminated vaccine *(1).*

Puntoni et al. suggest that the peak ependymoma incidence in 1964 could partly be due to SV40 infection acquired by children from their mothers as a result of vaccination during pregnancy. This interpretation is implausible for several reasons. First, the peak in incidence in 1964 was based on only seven ependymoma cases; thus, the single-year incidence estimate was unstable. When we smoothed the incidence data, the peak in incidence occurred in 1969. Second, no ependymoma cases were observed among 0- to 4-year-old children in 1962, even though mothers of all children followed in 1962 also could have received SV40-contaminated vaccine during pregnancy. Third, in the study *(3)* cited by Puntoni et al., children whose mothers had received poliovirus vaccine during pregnancy had an increased risk of cancer, but no child developed ependymoma. In addition, a subsequent serologic study found little evidence of SV40 infection occurring during pregnancy in vaccinated mothers *(4),* so the basis for the increased cancer risk in these children remains unknown.

Although we agree with Puntoni et al. that our results for mesothelioma and osteosarcoma were less conclusive, our study still provides relevant data on these cancer outcomes. Our data for mesothelioma were strongest for individuals aged 33 years or younger; however, although mesothelioma incidence increases most steeply for older individuals, the extreme rarity of mesothelioma in younger individuals illustrates the absence of an observable effect of SV40 infection on mesothelioma risk for three decades after childhood exposure. Furthermore, among older adults in the United States, Strickler et al. *(5)* found

no evidence for increased mesothelioma risk related to exposure to SV40-contaminated poliovirus vaccine. Finally, osteosarcoma comprises the majority of bone tumors in persons aged younger than 25 years *(1)*. Therefore, the similarity in incidence of combined bone tumors across birth cohorts argues against an increased incidence of osteosarcoma in Danish children who received SV40-contaminated vaccines.

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NOTES

Editor's note: Dr. Frisch is employed by Statens Serum Institut, the manufacturer of inactivated poliovirus vaccine used in Denmark since 1955.

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